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DETERMINATIONS OF IMIDACLOPRID AND NANO- IMIDACLOPRID ON SOME TOMATO SERIOUS PESTS AND THEIR PREDATORS

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ABSTRACT

The effect of the fungi Imidacloprid and Nano- Imidacloprid on the target insect pest show that, under laboratory condition the LC50s obtained 88.3x 104 and 127.3x 104conidia/ml for B. tabaci after treated with different concentrations of Nano-Imidacloprid and M.a respectively. The LC50s for M. persicae recorded 66.4x 104 and 137.2x 104 conidia /ml after treated with the corresponding pathogen.

Under field condition in El Esraa (Nobarya) the data obtained detect tat, the M. persicae significantly decreased to 7,1±4.6, 17.1±4.8and 27.5±4.8 individuals after post applications of Nano-Imidacloprid treatments after 50, 90 and 120 days. When B. tabaci treated with Imidacloprid the individuals significantly decreased to 25.5±4.4, 29.9±4.8, 39.9±8.8and 49.9±2.8 individuals as compared to 49.4±5.8, 80.4±5.8, 98.4±5.8 and 188.4±5.8 individuals in the control after20, 50, 90 and 120 days respectively. El-Kassaseen (Ismailia) governorate, both of the target insect pests significantly decreased.

The experiments in the field showed that, in two governorates, the tomato infesta tion with B. tabaci or M. psicae significantly decreased after treatments by Imidacloprid and nano-Imidacloprid as compared to control plants.

KEY WORDS: Bemisia tabaci, , whitefly, aphids, bioinsecticide toxin, tomatoes, Coccinella undecimpunctata.

INTRODUCTION

Tomatoes plants *(Lycopersicon esculentum*) among the highy important crop in all words and in Egypt. Tomatoes crops are infested with very important dangerous insects. The most harmful insect pests are the green peach aphid, *Myzus persicae*, and whitefly, *Bemisia tabaci*. These pests causes many viruses to the plants and causing great infestation and harmful disease to the leaves and fruits, also transfer the virus diseases (Namba and Sylvester 1981; Berry 1998; Filotos et al. 2004). The chemical pesticides, causing a pollution to the environment and causing a reduction in the beneficial insects. Also, they developing the insecticidal resistance among the insect pests and then, consequently, causes an inevitable outbreak (Lowery and Sears 1981). lately, many agriculture studies have favor to use of many microbial agents as a biological agents alternate. Opposite to the other microbial pesticides, bacteria, fungi which cause a successfully controlled against many insect pests (Sabbour and Shadia Abd El-Aziz 2002; Sabbour and Sahab 2005, 2007; Thungrabeab and Tongma 2007; Sahab and Sabbour 2011). The two entomopathogenic fungi, *Nomuraea rileyi* and *Isaria fumosorosea*, demonstrate a very higher pathogenicity against aphids and whiteflies (Espinel et al. 2008). The entomopatogenic fungus *N. rileyi*, decrease the host discriminatory infections against the lepidopterous larvae (Ignoffo et al. 1976). Shanthakumar et al. (2010) recorded that the entomopathogenic fungi *N. rileyi*, make a reductions to many pest insect. In this manner, James and Lighthart (1994) recorded that entomopathogenic fungi *Imidacloprid* infect many insects among the Coccinella. Farag (2008), recorded that entomopathogenic fungi *B. bassiana* could to infect *C. undecimpunctata* when sprayed at higher concentration.

The aim of our study to determine the efficacy of *Imidacloprid*and its toxin Nano-Imidacloprid against (*M. persicae* and *B. tabaci*) under laboratory and field conditions and on predator *C. undecimpunctat*a .

MATERIALS AND METHODS

Insect cultures

The target insects of Whitefly *B. tabaci* and *M. persicae* were reared under laboratory conditions 26±2°C and 65±5% RH on a potted tomato plants inside cylinder glass cages (16 cm in diameter x 45 cm in height. The cages were covered with muslin.

The Predator culture of *C. undecimpunctata*, were collected from tomato field which infested with aphid from, Giza governorate, Egypt. Glass jars (25) held 15 adults each. The jars were supplied with fresh duranta leaves infested with aphids for feeding. They were covered with muslin cloth which was held in position with rubber bands. Food was `renewed every other day. The experiment investigated every day inorder to count the eggs. The eggs were collected and then transferred to a Petri dishes (20 cm diameter) till hatching. The early new larvae counted and then transferred to a plastic jars till the larvae reached (2nd nymphal instar).

The jars contained and definite amount of eggs. Unused nymphs were left in 2 l glass jars (5/each) with small duranta branches carrying different stages of aphids, till maturation.

Source and production of fungi The fungi *Imidacloprid* and Nano-Imidacloprid were kindly obtained from Dr. Alain Vey (Prof.), Mycology Unit, Pasture Institute, France, and they produce by the team in the Microbiology Department, National Research Centre, Cairo, Egypt. They were primarily purified by using the mono-spore technique. Then, the fungi were propagated in Petri dishes (9 cm) on Potato Dextrose Agar medium (PDA) enriched with 1% peptone, 4% glucose, and 0.2% yeast extract and incubated at 26°C. Seven-day-old cultures with well developed conidia, were harvested by washing with 10 ml sterilized water. Then 3 drops of Tween-80 were added to 100 ml with water. It was used as stock suspension and kept refrigerated at 4°C. From this stock, dilutions with water were adjusted at the needed proposed concentrations. Large amounts of conidiospores, if needed, were produced by culturing the fungus on liquid medium in 1 l cell-culture glass bottles according to Rombach et al. (1988) (modified by El-Husseini et al. 2004).

Laboratory tests

The target insect pests n treated with entomopathogenic fungi *Imidacloprid* and the toxin Nano-Imidacloprid at the concentrations ranged between $1x10^2$ to $1x10^8$ conidia/ml. These concentrations were prepared by a dilutions by 1–10 fold from the main prepared culture tank and then used on the target insect pests B. *tabaci* and *M. persicae* at the 3rd nymphal instars. A fresh tomato leaves were sprayed with the last entomopathogenic fungi (3 shots as spurts/leaf) (Matter et al. 1993), then they will left to dry and then they put inside a plastic jars (one/each). Then, a twenty five nymphs of either species were placed on each leaf. The experiment replicated five times. The experiment carried out at 26±2°C and 65±5% RH. Each jar incubated at 25°C. The percentages of mortality were calculated after seven days and corrected according to Abbott, (1925), while LC50 was calculated through probit analysis according to Finney (1964).

Treatments of the predator One-day-old adults and 2nd instar nymphs of *C. undecimpunctata* were used for the evaluation of the pathogenicity and efficacy of *Imidacloprid* and toxin Nano-Imidacloprid spores.

The methods used:
A – Spray technique to evaluate contact effect.

B – Feeding technique in order to determine the oral toxicity, which required by meant there was no choice (there was exposure to those prey treated only), or free-choice which meant there was exposure to both infected and uninfected prey. This was done to see whether the predator had the ability to recognize infected from uninfected prey.

A – Spray technique There were 20 predators of one-day-old adults or one day-old 2nd nymphal stage predators per group. Each group was placed in a petri dish (25 cm diameter) and sprayed with the ***Imidacloprid*** or Nano-Imidacloprid at a concentration of $1x10^8$ (Matter et al. 1993). The shots were directed at the insects at a 15 cm distance. Then, make a tweezers to take the insects were to plastic jars (5 cm diameter and 15 cm height). The jars had a small water moistened filter paper and an aphid infested tomato plant leaf.

B – required and free-choice feeding techniques Groups of twenty individuals of either adults or 2nd instar nymphs per group were exposed to either a required contaminated diet (*Imidacloprid* or Nano-Imidacloprid treated aphids), or selectively to entomopathogenic fungi treated and untreated aphids, for 24 h. In the case of the free-choice feeding, five groups were used/pathogen/predator stage. The predator nymphes were starved for 4 and 6 hours for nymphes and the predator adults were starved for 6 h. Then, each group was introduced in the middle of 5 l jar in which had been placed two branches of tafla carrying ample amounts of the pests. One branch was previously sprayed with the fungus, while the other branch was sprayed with water only. The two branches were placed on both sides of the glass jar, facing each other, to allow the predator individuals free choice to feed on either the treated or untreated aphids. Five glass jars (replicates) were used/ each pathogen. Regarding the required feeding, the same number of predators in each of the 5 glass jars were used as mentioned above, but these predators were offered only treated aphids. In both trials, the exposure period was 24 h. Then, the predators from all the treatments as well as the control, were transferred individually to plastic cups, offered untreated aphids, and checked daily for 14 days.

Field experiments

The field experiments were made against the target insect pests in two places field . Each place area has different climatic and soil factors. These two areas were: (Nobaryia region) with a dry climatic weather and the sandy soil, the other place in (Ismailia)which have a wet climatic weather and have a clay soil. Tomato plants (Var. GS12) were planted in the 1st of April in an area measure about 2,400 m^2, divided into twelve plots of two hundreds m2 for each plot. About4 plots were applied with each pathogen, while 4 plots for the controls (untreated). *Imidacloprid* and Nano-Imidacloprid were applied at $1x10^8$ conidia/ml. Treatments were sprayed at a randomized plot at sunset. A ten-litre sprayer was used to spray on the treatments. Three applications were made at one week intervals, at the commencement of the experiment. Twenty plant samples were randomly collected at certain time intervals from each plot and transferred to the laboratory for examination. The average number of each of the tested pests/ sample/plot/treatment was cached at 20, 50, 90 and 120 days after the first application. The number of the infestation of the target insect pests were then calculated in each case. After harvest time, the yield of each pathogen treatment was weighed as kgs/feddan.

Predator Seedlings of tomato plants were sown in rows (Ca 50 cm from each other) in ca half a feddan located in the village of Manawat, Giza governorate. One-month-old plants were found highly infested with the *M. persicae* aphid, and white fly *B. tabaci*. The cultivated land was divided longitudinally into 3 areas (Ca Kirat/each), separated from each other by uncultivated land (4 m width). One area was used for each *M.a* or Nano-Imidacloprid and the control. Each pathogen was sprayed at the rate of 250 l/feddan, using a high pressure hand held gun. The concentration of the *M.a* or Nano-Imidaclopridwas about ($1x10^8$) conidia/ml. This concentration had previously achieved more than 80% mortality of both pests, in laboratory experiments. Three applications were made at one week intervals. Then, the *C. undecimpunctata* (nymphs and adults) were carefully scrutinized and counted, on site, in each of the treated and untreated tomato plant plots. The methods used were vision, hand picking, and also, a sweeping net (25 cm dimeter) was used. The counts were made just before the 1st application, and 1, 2, and 3 weeks after the last application. After each count, the predators were once again placed on their previous location at the corresponding plant site. Fifty tomato shrubs (10 each from 5 rows) per each treated area as well as the control, were arbitrarily chosen for each time interval. The average number of predators/50 plants/time interval was calculated in each case. The increase or decrease in the population density of the predator/50 plants as compared with the control, was calculated according to Henderson and Tilton's (1955) equation, as follows:

where: Ca – population density in the treated area before treatment Cb – population density in the treated area after treatment Ta – population density in the treated before treatment Tb – population density in the treated area after treatment.

RESULTS AND DISCUSSION

The effect of the fungi *Imidacloprid* and its toxin Nano-Imidacloprid on the target insect pest show that, under laboratory condition the LC50s obtained 88.3x 10^4 and 127.3x 10^4conidia/ml for *B. tabaci* after treated with different concentrations of Nano-Imidacloprid and *Imidacloprid* respectively. The LC50s for *M. persicae* recorded 66.4x $10^4$and 137.2x 10^4 conidia /ml after treated with the corresponding pathogen (Table 1).

Table 2 show that under field condition in (Nobarya) the data obtained detect tat, the *M. persicae* significantly decreased to 7±5.6, 17.1±4.8and 27.5±4.8individuals after post applications of Nano-Imidacloprid treatments after 50, 90 and 120 days. When *B. tabaci* treated with *Imidacloprid* the individuals significantly decreased to 25.5±4.4, 29.9±4.8, 32±8.2and 49.9±2.8individuals as compared to 49.4±5.8, 80.4±5.8, 98.4±5.8and 188.4±5.8individuals in the control after20, 50, 90 and 120 days respectively. (Ismailia) governorate, both of the target insect pests significantly decreased (Table 2).

Experiments which made inside the field in (Nobarya) and (Ismailia), show that, the infestation percentages of *B. tabaci* or *M. psicae* were significantly decreased in plots which treated with *Imidacloprid* or *nano-Imidacloprid*, as compared to control. Although the infestations of both of the two target insect pastes were decreased after bot pathogen treatments. (Table 2). Effects of *Imidacloprid* and Nano-Imidacloprid on *C. undecimpunctata* showed no effect . Under the laboratory investigations results showed that the predator *C. undeciumpunctaata* is less susceptible to all both of the two pathogens (Table 3). At all, predators adults showed greater resistance to both pathogen treatments. But the predator nymphs affect more than the adults. Under Laboratory conditions , results obtained that the percentages of the mortality in predators adults after *Imidacloprid* treatments spray was about 0.62 times which show a more in the mortality than percentages obtained from Nano-Imidacloprid treatments. (Table 4). Treatments of *Imidacloprid*, showed a highly reductions in the population densities (63.33 and 43.99%) were estimated in the 1st and 2nd weeks after the last application, respectively.

At the end of the experiments, the weight of tomatoes showed an significantly increase to 2991± 84.31 and 2921± 34.31 Kg/feddan in plots treated with Nano-Imidacloprid and Imidacloprid respectively as compared to 1989± 34.31 Kg/feddan in the control in (Nobarya) (Table4). In (Ismailia) the weight of tomatoes crops significantly inccreased to 2979±75.88and 2704±75.88 Kg/feddan in plots treated with Nano-Imidacloprid and *Imidacloprid*, respectively as compared to 1700±75.8881Kg/feddan in the control.

The same obtained agree with Sabbour and Shadia Abd El-Aziz (2002 and 2010). Our results agree with Abdel-Rahman and Abdel-Mallek (2001), AbdelRahman (2001) and Abdel-Rahman and Abdel-Mallek (2001), Abdel-Rahman et al. (2004, 2006) who reported that, the bioinsecticided control all of the cereal aphids especially the entomopathogenic fungi. The same finding obtained by, Sabbour and Sahab (2005, 2007), Sahab and Sabbour (2011) found that fungi control many of insect infested the cabbage and tomato crops under laboratory and field conditions. Our results showed that the predetor *C. undeciumpunctata* not affected with the tested fungi M.a, and Destruxin. Goettel et al. (1990) reported that the fungi commercial formulations controlled aphids and thrips with low in the influence impact on non-target insects. The same findings with Todorova et al. (1994), who recorderd that of *B. bassiana* different strains cause an effects on the two predatory insects due to the host response of the insects. Poprawiski et al. (1998) found that *Serangium parcestosrum* (Coccinellidae) had lower survival potential when sprayed with *B. bassiana* fungus than when sprayed with *P. fumosoroseus* fungus. Shanthakumar et al. (2010) considered that despite the great virulence of *Imidacloprid*against *S. littura*, the pathogen proved reasonably safe for *T. chilonis. Imidacloprid*did not cause reduction in the parasite percentages of *T. chilonis*. The present results also indicated that the predator, *C. undeciumpunctata,* particularly the adult predators, can distinguish between fungus-infected prey and non-infected prey. *C. undeciumpunctata* will almost always avoid the treated prey, especially if given free-choice feeding. This, however, was more pronounced in the case of Detruxin than *Imidacloprid* . This phenomenon observed in our investigations was also noted by many other authors. It was mentioned that predators, when given free choice to feed on fungus – treated or untreated aphids, predation on the infected prey was less than predation on the uninfected ones (Baverstock et al. 2007). Also, Roy et al. (2010) and Goettel et al. (1990) proved that *C. septempunctata* adults avoid contact with leaf and soil surfaces innoculated with *B. bassiana* fungus and mycosed cadavers. The predator was more often positioned away from mycosad cadaver than from uninfected ones. Neverthless, some researches indicated several adverse effects of some entomopathogenic fungi against some natural enemies. Haseeb and Murad (1997) and Delete et al. (1995) consider *C. septempunctata* to be somewhat susceptible to *B. bassiana*. While, Farag (2008) consider that some entomopathogenic formulations of *B. bassiana* have deletorious effects on *C. undecimpunctata* if applied at high concentration levels. The various views about the safety of entomopathogenic fungi stated by many different authors, might be due to the relative efficacy of the fungus or its isolates on pests which exhibit different susceptibilities, bionomics, and characters. The various views may also be due to the types of assessment and application rates. The same results obtained by (Sabbour and Hany a&b, (2007), Mattr and Sabbour 2013,Sabbour, 2007 who found tat the usages of the bio insecticides not affect on the parasites and predators. Also Sabbour and Nayera 2016, a&b controlled the locust and grasshopper with bio pesticides. Sabbour and Singer 2016, a&b control insects by *Imidacloprid* and nano- *Imidacloprid*. Sabbour and Abd El Raheem 2016, Sabbour and Hussein 2016 found that bioinsecticides not pollute the environments.

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Table 1: The effect Imidacloprid and its nano- Imidacloprid against *B. tabaci* and *M. persicae* under laboratory.

Insects	Treatments	LC_{50}	95% confidence limits
B. tabaci	Nano-Imidacloprid	88.3x 10^4	70.7 – 120
	Imidacloprid	127.3x 10^4	90 – 135
M. persicae	Nano-Imidacloprid	66.4x 104	61 – 139
	Imidacloprid	137.2x 10^4	89 – 159

Table 2. *B. tabaci* and *M. persicae* infestation after treatment with the pathogen under field conditions.

Treatments	Days after 1st application	(Nobarya)		(Ismailia)	
		B. tabaci	*M. persicae*	*B. tabaci*	*M. persicae*
Control	20	55±1.1	54±1.6	55±1.1	64±3.5
	50	77±1.9	72±9.1	80.4±5.8	187±3.2
	90	87±9.6	97±3.3	98.4±5.8	198±9.7
	120	163±4.8	150±7.0	188.4±5.8	165±6.7
Imidacloprid	20	25.5±4.4	13±4.7	12±5.3	9±3.9
	50	29.9±4.8	18±2.8	35±6.6	27±4.3
	90	39.9±8.8	33±4.2	43±4.2	36±4.1
	120	49.9±2.8	41±7.1	53±6.9	49.4±5.8
Nano-Imidacloprid	20	0	0	4±0.3	5±0.2
	50	11±1.6	7,1±4.6	12±1.5	10±1.7
	90	22±2.8	17.1±4.8	20±4.9	21±3.4
	120	30±8.5	27.5±4.8	35±5.8	36±5.7
F test Lsd 5%		25.3 11.2		24.7 13.5	

Table(3): effect of the Imidacloprid and nano- Imidacloprid on developmental stages of C. Undeciumpunctata.

Pathogen	percentages of th mortality of infected insects					
	Adults			Nymphs		
	Type of treatment					
	Direct spray	Ingetion treated diet		Direct spray	Ingestion treated diet	
		Obligatory	Selection		Obligatory	Selection
Imidacloprid	19.20 ± 7.61	18.4 ± 2.71	7.2 ± 1.5	28.6 ± 2.19	29.6 ± 3.49	12.8 ± 1.96
Nano-Imidacloprid	31.2 ± 4.45	33.6 ± 4.49	24.8 ± 2.82	41.6 ± 3.71	39.2 ± 4.45)	22.4 ± 2.40
F test Lsd 5%	28.9 10.9					

Table (4): Average number of *C. undecimpunctata* (all stages) / 50 tomatos shrubs after successive post fungal application period.

post application (Weeks)	Treatments Average number of C. undeciumpunctata ± SE				
Just before 1st. Application	Control	Imidacloprid	Nano-Imidacloprid	% increase(+) or decrease (-)**	
Weeks after last Application	18.75± 3.36	21.00± 2.55	20.00± 1,58	Destruxin	M.anisoplia e
One week after the last last Application	21.25±1 .70	16.11±2.15	7.3±1.71	-36.22	-69.09
Two weeks after the last last Application	19.20±1 .50	17.11±1.16	11.5±0.96	-19.81	-43.99
Three weeks after the last last Application	17.00±1 .12	20.15±8.65	14.20±8.93	+6.36	-21.42

Percentages of increase or decrease in C. undeciumpunctata population denesity as compared with the check according to Hendrson and Tilton (1955).

Table (5): Tomatoes weight after pathogens tested on B. tabaci and M. persicae in

	(Nobarya)	(Ismailia)
Treatments	Weight tomatoes (Kg/feddan)	Weight tomatoes (Kg/feddan)
Control	1791± 84.31	1700±75.88
Nano-Imidacloprid	2991± 84.31	2979±75.88
Imidacloprid	2407± 12.17	2704±75.88
F values	31.02	32.43
LSD 5%	81	82

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